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Source: Journal of Wildlife Diseases, 7(3) : 166-170

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-7.3.166

SEROLOGIC EVIDENCE OF VENEZUELAN EQUINE ENCEPHALITIS VIRUS INFECTIONS IN RACCOONS OF SOUTH CENTRAL FLORIDA

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Abstract: A presumed second human case of Venezuelan equine encephalitis (VEE) approximately 175 miles north of the Everglades National Park, prompted an epizootiological survey to document the presence and distribution of VEE virus in small mammals throughout the state. Sera from 339 small mammals from 25 counties in Florida were tested for hemagglutination-inhibiting antibodies against VEE, eastern encephalitis (EE) and St. Louis encephalitis (SLE) viruses. Sera reactive to VEE in HI tests were also examined for neutralizing antibody to VEE and EE. Significant HI and neutralizing antibody to VEE virus was detected in raccoons *(Procyon lotor)*, cotton rats *(Sigmodon hispidus)*, cotton mice *(Peromyscus gossypinus)* and an opossum *(Didelphis marsupialis)* in various habitats along the Gulf and Atlantic Coasts of Florida. These data indicated that (1) VEE virus may be widespread in south central Florida; perhaps endemic in Indian River County (2) Raccoons may be excellent indicators of VEE activity in a statewide arbovirus monitoring system.

INTRODUCTION

In recent years, there has been considerable interest in wild mammals, particularly rodents, as possible reservoir hosts of Venezuelan equine encephalitis (VEE) in south Florida. Since the first naturally occurring human infection was detected October, 1968 in Homestead,[1,4,5] the Florida Division of Health (FDH) has conducted an intensified statewide VEE surveillance program. In November, 1968 a 71 year old male became ill with central nervous system disturbances after exposure to mosquitoes outside his trailer residence near Sebastian, Indian River County. The Virology Section of the FDH Bureau of Laboratories, subsequently demonstrated a serologic rise in antibody titer to VEE virus.[4] Since the presumed site of infection was approximately 175 miles north of Homestead, it was deemed important to document the presence of VEE virus in the area via the selective sampling of small mammals. The study was subsequently expanded and small mammals collected throughout the state of Florida were surveyed for the presence of VEE antibody. The results of these studies are presented in this report.

METHODS AND MATERIALS

During the initial investigation in July 1969, wild vertebrates were collected from citrus groves, fallow fields, woodlots, ditchbanks and a city dump within a one mile radius of the patient's vacation trailer. A follow-up investigation one month later intensively sampled mammals of the sandy ridge, oak-pine-palmetto habitat, and areas along the Indian River and Sebastian Creek within a three mile radius of the trailer. Selected rodents were also collected from a citrus grove and tropical hammock approximately 15 miles south in the vicinity of Vero Beach.

In an attempt to delineate the geographic distribution of VEE virus activity on a statewide scale, raccoons, opossums and certain rodents were collected from state parks and private tracts in 24 counties. Trapping operations commenced in November 1969 and continued for one year. All animals were captured alive in cage, box or steel traps, anesthetized and bled by cardiac puncture. Blood samples less than 2.0 ml were diluted with buffered saline as described previously.[1] Hemagglutination-inhibition (HI)

tests were performed by the FDH Bureau of laboratories' Virology section with VEE, eastern encephalitis (EE), and St. Louis encephalitis (SLE) antigens according to a modification of the method described by Clarke and Casals.[3,4] Sera with VEE HI titers 1:10 or greater were submitted to the Center for Disease Control (CDC) Arbovirus reference laboratory, Atlanta, for confirmatory neutralization testing. Two or four fold serum dilutions were tested in primary duck embryo cell cultures for neutralizing antibody against New Jersey strain of EE (NJO) and the Ft. Detrick vaccine strain of VEE (TC-83). In some instances selected sera were also tested against the Florida strain of VEE (Fe3-7C).

RESULTS

During the initial investigations 94 small mammals of six species were collected from Indian River County. VEE HI antibodies were detected in sera from four of ten raccoons *(Procyon lotor)* and four of 44 cotton rats *(Sigmodon hispidus)* trapped within three miles of the patient's residence (Table 1). All four raccoon sera and two of the four cotton rat sera reactive in the HI test produced significant titers in neutralization tests. HI reactive sera from one cotton rat and one cotton mouse *(Peromyscus gossypinus)* trapped in a hammock west of Vero Beach were also confirmed in neutralization tests (Table 2).

The HI results of mammal sera collected during the statewide survey are presented in Table 3. Only seven of 245 sera were reactive for VEE in the HI test. Five of these were from a group of six raccoons collected in Johnathan Dicksinson State Park, Martin County (Figure 1). A sixth HI reactive raccoon came from Hobe Sound, also in Martin County along the Atlantic coast. The remaining HI reactive sera came from an opossum collected 8 miles north of Naples, Collier County on the Gulf coast.

Table 1. VEE HI antibody rates in small mammal sera collected in Indian River County, July and August, 1969.

Species	1 Mile*	2 Miles	3 Miles	15 Miles Grove	15 Miles Hammock	Totals
Cotton Mouse *(Peromyscus gossypinus)*			0/1		1/2	1/3 (33.3%)
Cotton Rat *(Sigmodon hispidus)*	3/30	0/5	1/9	0/13	1/2	5/59 (8.6%)
Black Rat *(Rattus rattus)*	0/4					0/4
Raccoon *(Procyon lotor)*	2/7		2/3			4/10 (40%)
Opossum *(Didelphis marsupialis)*	0/8	0/2	0/7			0/17
Gray Fox *(Urocyon cinereo-argenteus)*	0/1					0/1
Totals	5/50	0/7	3/20	0/13	2/4	10/94

* Airline distance from Patient's residence

Table 2. Comparison of VEE hemagglutination — inhibition and neutralization antibody levels in mammal sera collected from Indian River County.

Area and Species	HI Titer[2] VEE	HI Titer[2] EE	N Titer[3] VEE	N Titer[3] EE
One Mile[1]				
Cotton Rat	40	<10	64	<4
Cotton Rat	40	<10	<4	<4
Cotton Rat	10	<10	<4	<4
Raccoon	40	40	64	<4
Raccoon	40	<10	64	<4
Three Miles				
Cotton Rat	10	<10	4	<4
Raccoon	40	<10	≥128	<4
Raccoon	40	<10	64	<4
Fifteen Miles				
Cotton Rat	20	<10	32	<4
Cotton Mouse	40	<10	8	<4

[1] Airline distance from patient's residence
[2] Reciprocal of serum dilution
[3] Reciprocal of highest serum dilution that produced at least 90% plaque reduction

Table 3. Summary of small mammal sera tested for Venezuelan equine encephalitis HI antibodies during statewide survey in Florida 1969-1970.

County	Raccoon	Opossum	Cotton rat	Cotton mouse	Other
Escambia					0/12[1]
Okaloosa	0/1	0/2			
Bay	0/4				
Jackson	0/1	0/1			
Liberty	0/2	0/1			
Suwannee	0/5	0/7	0/26	0/6	
Columbia	0/1	0/16			
Levy	0/5	0/8			
Nassau	0/12	0/17			0/1[2]
Clay	0/5	0/5			
St. Johns	0/8				
Marion	0/2				
Volusia	0/5	0/3			
Lake	0/1	0/6			
Hardee	0/1				
Hillsborough	0/5				
Polk	0/3	0/16	0/4		
Sarasota	0/7	0/1			
Duval	0/2	0/1			
Brevard		0/3			
Highlands	0/15				
Martin	6/9	0/2			
Collier	0/3	1/4			
Monroe	0/6				
Totals	6/103	1/93	0/30	0/6	0/13

[1] Striped Skunk
[2] Cottontail Rabbit

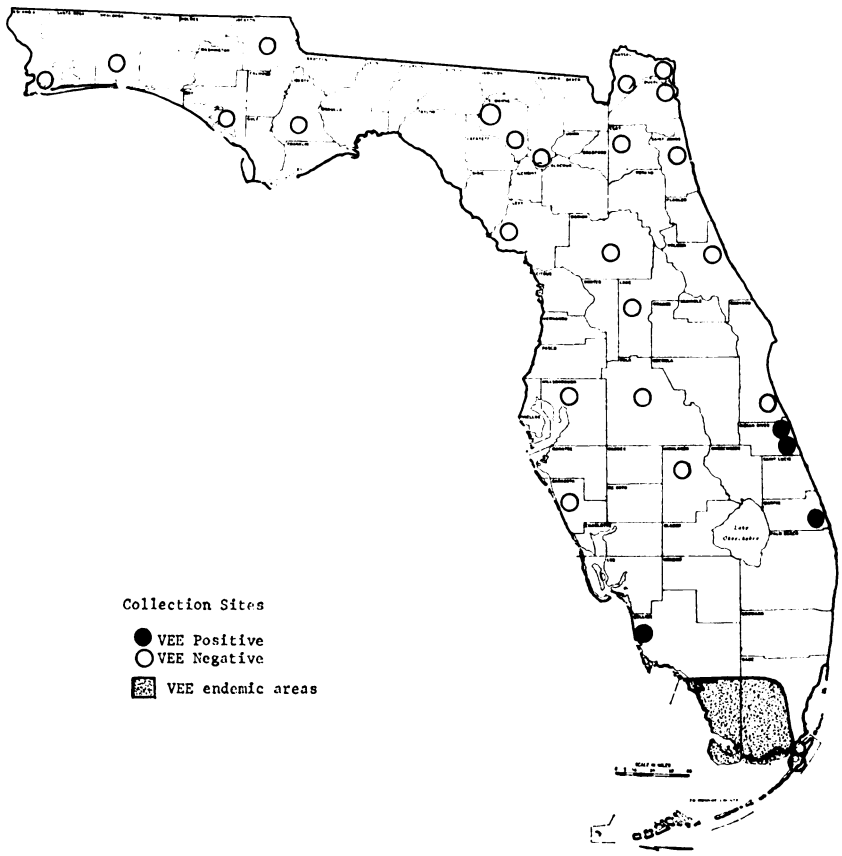

Figure 1. Distribution of VEE HI and neutralizing antibodies in small mammals collected in Florida 1969-1970.

All seven sera were confirmed in neutralization tests at CDC, although the opossum sera only neutralized the Florida strain of VEE and not the Ft. Detrick strain.

DISCUSSION

The demonstration of VEE HI and neutralizing antibodies in sera from small mammals collected from Indian River County documents activity of this virus in an area approximately 175 miles north of the Everglades endemic areas.[1,2] The naturally acquired human infection from Sebastian appears to have coincided with the case at Homestead in 1968.[4] The epidemiologic investigation in south Dade County revealed significant VEE antibodies in four of five species of rodents. The agricultural land, sawgrass marsh and tropical hammocks in the vicinity of Florida City - Homestead, tend to support large natural populations of rodents. In contrast, the oak-pine-palmetto habitat of the Sebastian area does not support large rodent populations. Most of the cotton rats trapped within three miles of the patient's trailer residence were obtained along the edges of Sebastian Creek, the Indian River and associated drainages. Significant VEE

antibody titers from two cotton rats and four raccoons trapped within this limit would seem to indicate circulation of this agent in the recent past. The high infection rate found in cotton mice and cotton rats from a hammock west of Vero Beach is indeed interesting. Ongoing studies may indicate an endemic foci of VEE virus in Indian River County.

The high prevalence of VEE antibodies in raccoons from Johnathan Dickinson State Park and Hobe Sound, also coastal sandy ridge habitats, suggests that VEE virus is not necessarily confined to wild rodent-mosquito cycles outside of the Everglades area. Raccoons may someday prove to be an important vertebrate host serving to disseminate VEE virus along major drainages and coastal areas. In any case, they may be excellent indicators of VEE activity in a statewide arbovirus monitoring system.

Acknowledgements

The author expresses appreciation to Mrs. Ellnora Lassing for her technical expertise in performing the hemagglutination-inhibition tests and to Mr. David Sikes, Mr. Hardrick Gay, and Dr. Herbert Kale for their assistance during the field investigations. Grateful acknowledgement is extended to Dr. Adrian Chapell, Center for Disease Control, Atlanta, Georgia for providing confirmatory neutralization testing services.

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Received for publication February 8, 1971